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# Ets-1 Flips for New Partner Pax-5

Minireview

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**Protein partnerships provide specificity for transcription factors that display conserved DNA binding properties. The newest Ets-1 partner Pax-5 directs a surprising conformational change that maximizes the protein interface and changes binding site preference.**

## The Specificity Conundrum

A difficult problem in biology is the issue of specificity of action by molecules that in sequence and structure are almost identical. This problem is well illustrated by families of evolutionarily conserved DNA binding proteins, particularly transcription factors that need to activate and repress unique sets of target genes. A common pathway to specificity is a protein partnership in which two proteins bind DNA together, displaying added affinity and expanded sequence requirements. Although there are many examples of this strategy, only a few have been described in sufficient detail to build mechanistic models. Furthermore, analyses have rarely revealed how a partnership can be selective for a subset of family members. It is just such an example that was described in the December 2001 issue of *Molecular Cell*. Garvie and colleagues investigated the ternary complex of ETS domain protein Ets-1 and the Paired domain protein Pax-5 bound to DNA. Although the DNA binding domains of these classes of proteins are understood in terms of sequence-specific DNA binding, the new crystal structures decipher an interplay between partners that dramatically changes the binding site preference of Ets-1. The key mechanistic feature is a conformational change in Ets-1 that accompanies DNA binding only in the presence of Pax-5.

## ETS Proteins Bind DNA in Partnerships

The *ets* gene family provides an excellent example of the specificity conundrum [1, 2]. This family, which is found throughout the metazoa, has 25 members identified in the human genome, as well as eight and ten members in the *Drosophila* and *C. elegans* genomes, respectively. The family is implicated in a variety of biological pathways that regulate cell growth, differentiation, and apoptosis. In addition, ETS proteins contribute to developmental regulation as well as aberrant control of gene expression in human disease. The *ets* genes are recognized by conservation within the sequences that encode the DNA binding domain, termed the ETS domain. This 85-residue domain binds to the core consensus sequence 5′-GGAA/T-3′. There is also remarkable conservation in five additional flanking base pairs, as evidenced by selected consensus (SAAB) experiments performed on 12 different ETS proteins. Thus, targeting individual ETS proteins to particular genes is a challenging task.

Structural studies of five ETS proteins in complex with DNA, including PU.1, GABPα, Elk-1, SAP-1, and Ets-1, as well as biochemical analyses have provided a detailed description of the ETS domain-DNA interface (Figure 1) [3– 9]. The ETS domain bears a winged helix-turn-helix motif, which consists of three α helices and a four-stranded β sheet (wing). Residues from the recognition helix (H3) directly contact base pairs within the core consensus sequence. Interestingly, sequence preferences span nine base pairs, with those outside of the core contributing to indirect readout in which sequence dictates DNA conformation that is recognized in turn by protein-phosphate contacts [6, 10]. There is some distinction between family members, even within the recognition of the core sequence. For example, some ETS proteins do not bind equally well to A:T or T:A at position 9 [7]. Analyses of binding sites that diverge from the consensus should uncover additional unique properties of different ETS proteins. It is one such nonconsensus site that led to the discovery of the Ets-1/Pax-5 partnership on the promoter of *mb-1*, a gene that is expressed specifically in early B cell development [11].

Three ETS protein partnerships illustrate diverse strategies that enhance the affinity and specificity of ETS domain DNA binding (Figure 2). In the first, two subunits of the ETS protein GABPα bind with two subunits of GABPβ to form a heterotetramer. The β subunit, which provides a dimeric interface (data not shown) for the tetramer, does not itself bind DNA, but contacts several parts of the α subunit within or near the ETS domain [5]. This partnership enhances specificity by enabling the use of two tandem ETS binding sites. In a second example, the ETS protein SAP-1 binds with a homodimer of Serum Response Factor (SRF), with each protein making sequence-specific DNA contacts to conserved recognition sequences [12]. SRF contacts both the ETS domain and the "B box," which is separated from the DNA binding domain by a flexible linker. In the third example, which is the focus of this commentary, Ets-1 binds with a second DNA binding protein, Pax-5 [9]. In contrast to other partners, Pax-5 recruits Ets-1 to a binding site that displays a nonconsensus core sequence.

## Pax-5 Partnership Selects a Subset of ETS Proteins

Cooperative DNA binding by Pax-5 and Ets-1 requires close apposition of two binding sites to facilitate the formation of several protein contacts (Figures 2 and 3). In this network, the glutamine residue, Gln-336, from a loop preceding H1 of the ETS domain contacts Gln-22 of Pax-5. Tyr-395 of Ets-1 emanates from the recognition helix, H3, and contacts this same Pax-5 glutamine. A salt bridge formed by two additional H3 residues, Asp-

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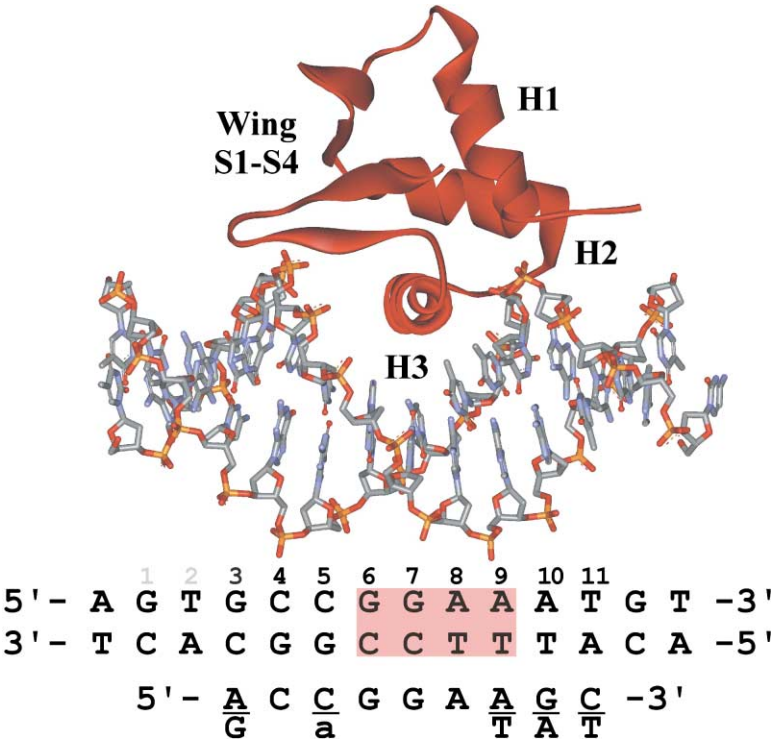


Figure 1. ETS Domain—DNA Interaction

Amino acids 331–415 of Ets-1 and numbered duplex [9]; positions 3–11 show sequence preference. Highlighted positions 6–9 define the core recognition sequence. Lower sequence is "selected" consensus sequence for Ets-1 [3].

398 and Lys-399, stabilizes this interaction. Asp-398 also interacts with a residue from Pax-5, Arg-56. A third Pax-5 residue, Leu-23, solidifies the interface by making hydrophobic contacts. The tyrosine homologous to Tyr-395 is highly conserved among ETS proteins; however, the other residues that are in contact with Pax-5 are less conserved. Indeed, only four of the 25 vertebrate ETS proteins contain the four crucial residues (Gln-336, Tyr-395, Asp-398, and Lys-399). Thus, the network at the Pax-5/Ets-1 interface can be formed by only a small subset of ETS proteins.

The network between Ets-1 and Pax-5 requires a subtle but significant conformational change in the side chain of Tyr-395 (Figure 4). Garvie and colleagues were able to detect this change by comparing the ternary complex to high-resolution structures of Ets-1 in the absence of Pax-5 on the *mb-1* promoter as well as on an ETS consensus site [9]. In the binary complexes, the Tyr-395 side chain extends into the major groove and forms a hydrogen bond to the base pair at position 8 or 9. This DNA binding role of the tyrosine side chain is similar to that observed in GABP and SAP-1. In striking contrast, Tyr-395 acquires a different position to form a hydrogen bond with Gln-22 of Pax-5 in the ternary complex. Importantly, the Asp-Lys salt bridge, which is formed by nonconserved residues, stabilizes the altered position. Indeed, SAP-1, which does not have the homologous aspartic acid to form the salt bridge, does not partner with Pax-5 [13, 14]. Thus, tyrosine, a residue conserved in almost all ETS proteins, is directed to perform alternative functions by interactions with nonconserved residues.

## Conformational Change Enhances DNA Binding Specificity

The altered position of Tyr-395 favors recognition of the *mb-1* promoter that contains an optimal binding site for Pax-5 and a suboptimal site for Ets-1. The 5′-GGAG-3′ core sequence of the *mb-1* promoter diverges from the 5′-GGAA/T-3′ consensus for ETS domain DNA binding. Indeed, the divergent G:C at position 9 lowers Ets-1 affinity 100-fold [13]. On the other hand, the G:C at position 9 is essential for Pax-5 DNA binding. In the binary complexes, the hydroxyl of Tyr-395 forms a hydrogen bond with base pairs at position 8 or 9. In the ternary complexes, Tyr-395 forms a hydrogen bond with Pax-5, rather than DNA, and van der Waals interactions are optimized by proximity with the C at position 10. In this way, the alternate conformer of Tyr-395 avoids the unfavorable G:C base pair and provides a new DNA contact for Ets-1. The net result is high affinity for a binding site that is unlikely to be used by Ets-1 alone. Thus, a conformational change in a single amino acid can dictate binding site preference for a nonconsensus core sequence.

The use of Ets-1 Tyr-395 as a sequence determinant is reminiscent of a phenomenon reported for the ETS protein Elk-1. In a crystallographically-derived structure of Elk-1 on DNA, the homologous tyrosine is directed away from the core sequence 5′-GGAA-3′ into a position strikingly similar to that observed in the Ets-1/Pax-5 ternary complex (Figure 4) [7]. The alternate position is stabilized in part by an Asp-Lys salt bridge homologous to the one observed in the Ets-1/Pax-5 ternary complex. It is proposed that this tyrosine conformer prevents Elk-1 from accommodating the A/T degeneracy in the core consensus, thus directing Elk-1 to prefer sites with a 5′-GGAA-3′ core sequence. Again, less well-conserved residues within the ETS domain dictate the conformational options of the tyrosine and, therefore, provide the specificity. In combination, the Ets-1 and Elk-1 examples demonstrate that conformational flexibility can add specificity to both binary and ternary complexes.

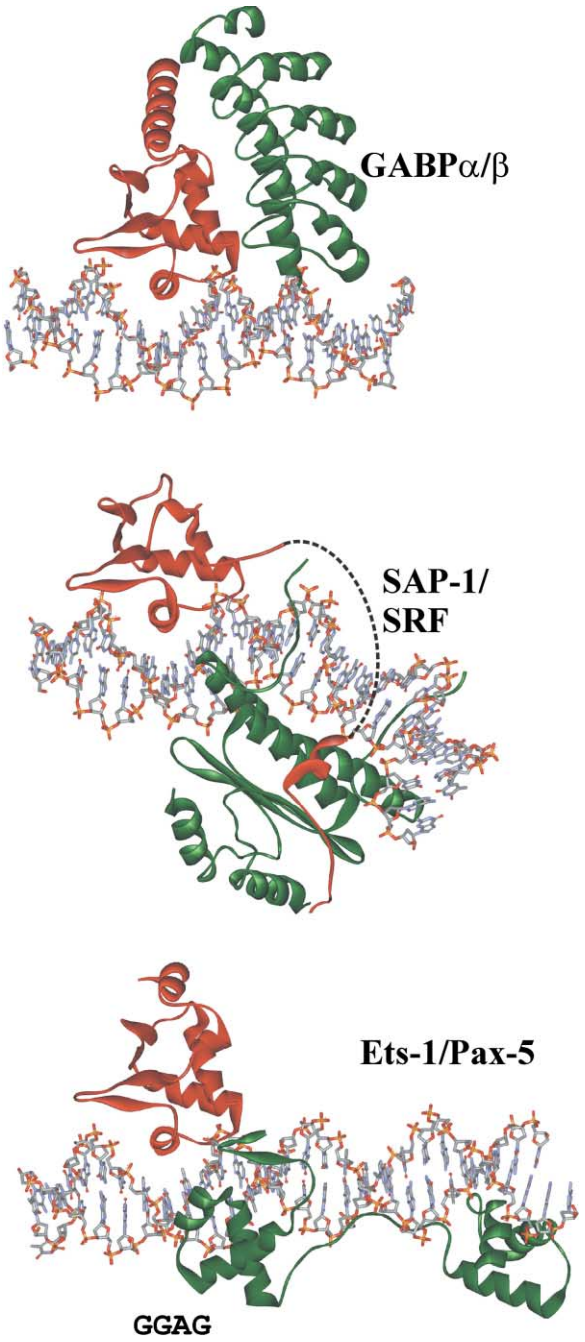


Figure 2. ETS Proteins Partnerships

DNA binding domains in complex with DNA as determined by crystallographic analyses: GAPBα with β subunit [5]; SAP-1 with SRF [12]; and Ets-1 with Pax-5 [9].

DNA binding of Pax-5 with Ets-1 illustrates that ternary complexes do not involve simple three-way docking. Rather, the interface can include new conformations that are not stably present in the absence of the partner. This theme of conformational change is played out in several other ETS protein partnerships. SAP-1 as well as Elk-1 interact with SRF via the "B box." This domain in SAP-1 is observed to be structured in the presence of SRF (Figure 2) but predicted to be unstructured in the absence of SRF [12]. Another interesting conformational

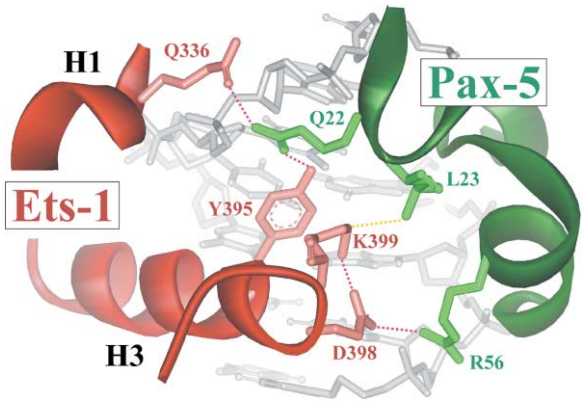


Figure 3. Network of Interactions at Interface between Ets-1 and Pax-5

Adapted from Figure 3A [9]; hydrogen bonds (pink) and hydrophobic interactions (gold).

change is the DNA-induced unfolding of an α helix that lies outside of the ETS domain of Ets-1 [15]. The elements involved in this structural transition, which are conserved only in Ets-1 and Ets-2, have an inhibitory effect on Ets-1 DNA binding, which is counteracted by a partnership with the DNA binding protein CBFα2 (RUNX1) [16, 17]. In these examples of both folding and unfolding transitions, nonconserved regions of the ETS proteins are utilized, providing a simple route to specificity.

In conclusion, ETS proteins participate in a variety of partnerships that provide specificity. Each partnership can direct subsets of ETS proteins to bind specific target genes and participate in distinct biological regulatory pathways. The targeted promoter must have the sequence-specific binding site for the partner as well as the core of an ETS consensus site. The Ets-1/Pax-5 complex demonstrates that even a minimal 5′-GGA-3′ core can be sufficient for recruitment of an ETS protein. Partnerships, therefore, diversify the sequence requirements for ETS protein targeting. The use of divergent regions outside of the ETS domain or even nonconserved residues of the ETS domain, as seen in the Ets-1/Pax-5 complex, ensures that only a subset of the family members will participate in any particular partnership. Finally, conformational flexibility provides an added level of control. The Ets-1/Pax-5 structures emphasize the potency of even a minor conformational change to dictate partner choice. These mechanistic strategies are likely to be used in the partnerships formed by other families of DNA binding proteins. The diversity in routes to specificity suggests that multiple levels of regulation will guide each transcription factor to its target gene. The choice of a partner is an important decision.

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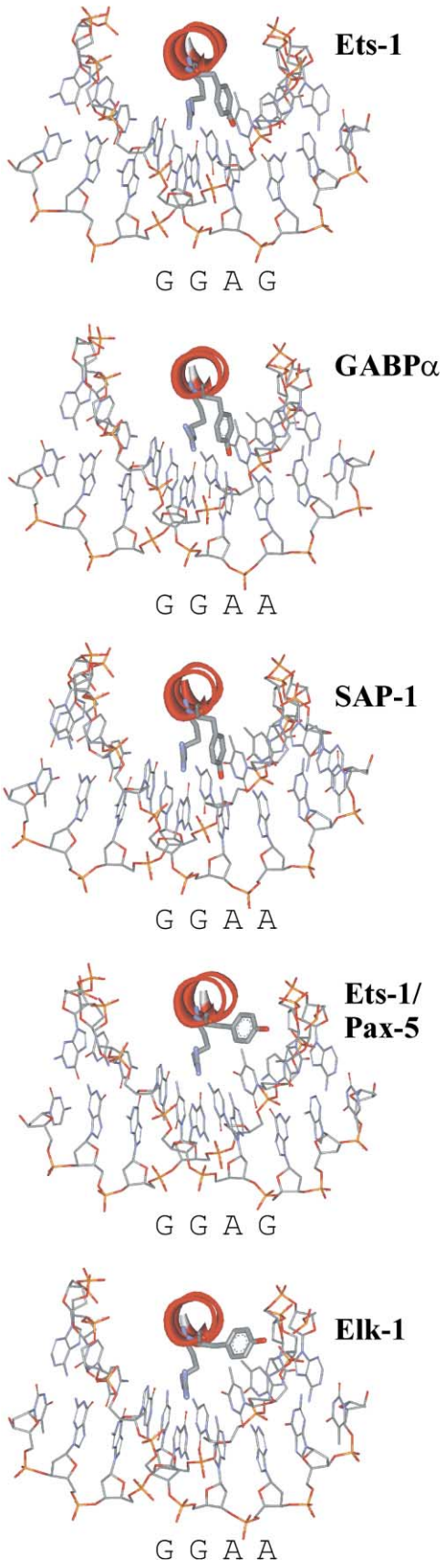


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Figure 4. Tyrosines Conformers among ETS Proteins

Ets-1 tyrosine 395 and arginine 391 from recognition helix H3 on duplexes with GGAA or GGAG (+Pax-5) core sequences. Homologous residues from GABPα [5], SAP-1 [6], and Elk-1 [7] bound to duplexes with GGAA core sequence.